# FINNISH HEREDITARY RETINAL DISEASES AND THEIR GENETIC BACKGROUND

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Summary

Hereditary retinal disases affect retinal photoreceptor cells and cause loss of vision and visual impairment. In Finland, hereditary retinal diseases are the leading cause of visual impairment in working age population and there are about 5,000 affected patients. These retinal diseases form both clinically and genetically heterogeneous group, but typically their onset age is low and progressing time long. Retinitis pigmentosa is the most common type of hereditary retinal disease. Cohen and Usher syndromes include the symptoms of retinitis pigmentosa, and are a part of Finnish disease heritage. Other Finnish heritage diseases are X-linked retinoschisis and choroideremia. Our aim was to study the prevalence, distribution and genetic background of hereditary retinal diseases in Finland. In addition, we reviewed stem cell-based therapy studies for retinal degenerative diseases worldwide.

Our observational study was based on Register of Visual Impairment (by THL, est. 1983) and Register of Retinal Diseases (by Retina Ry. est. 2016). The coding format of Register of Retinal Diseases was modified in order to standardise it and make the two registers comparable. The study population in Register of Visual Impairment was 1,831 and 83 in Register of Retinal Diseases. The analyses were performed using Microsoft Excel and computing environment R. The literature review was conducted using electronical databases MEDLINE and PubMed.

The high prevalence of working age registrants with hereditary retinal disease was seen in both registers. In Register of Visual Impairment, 50 % of the male patients were aged 42-67 years and the corresponding ages for female patients were 49-71. In Register of Retinal Diseases 50 % of all registrants were aged 38-51. In Register of Visual Impairment, 25 % of the male patients had disease onset age ≤17 years, ≤25 years in females. In Register of Retinal Diseases, the reported onset age of colour vision loss was low. Retinitis pigmentosa was a predominant diagnosis in both studied registers. The highest prevalence of hereditary retinal disease patients was in Satakunta (0,051 %, n=114). Only Register of Retinal diseases included information on genetic mutations. Mutations were most common in CERKL and EYS genes. To date, there are no regenerative therapy options for retinal degenerative diseases. However, clinical trials based on stem cells are going on. Future research based on genetic mapping and accurate coding into the national register systems could help to identify suitable cases for further in vitro and clinical studies.

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JOUKAINEN ELLI: SUOMALAISET PERINNÖLLISET VERKKOKALVOSAIRAUDET JA NIIDEN GEENITAUSTA

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Tiivistelmä

Perinnölliset verkkokalvosairaudet vaikuttavat verkkokalvon aistinsoluihin aiheuttaen näkökyvyn heikkenemistä ja näkövammaisuutta. Suomessa nämä sairaudet aiheuttavat eniten näkövammaisuutta työikäisillä ja potilaita on arviolta 5 000. Sairausryhmä on sekä kliinisesti että geneettisesti moninainen, mutta usein oireet alkavat nuorella iällä ja etenevät hitaasti. Yleisin verkkokalvon rappeumasairaus on retinitis pigmentosa. Suomalaiseen tautiperintöön kuuluvat mm. X-kromosomaalinen retinoskiisi ja korioideremia sekä Cohenin ja Usherin syndroomat. Cohenin ja Usherin syndroomat sisältävät retinitis pigmentosan oireet. Tutkimuksemme tavoite oli selvittää perinnöllisten verkkokalvosairauksien esiintyvyyttä, jakaumaa ja taustalla olevia geneettisiä muutoksia Suomessa. Lisäksi tutkimme verkkokalvon kantasoluhoitojen tämänhetkistä tilaa maailmanlaajuisesti.

Havainnoivan tutkimuksemme aineistona käytimme THL:n Näkövammarekisteriä (perustettu 1983) ja Retina Ry:n Retinitisrekisteriä (perustettu 2016). Retinitisrekisterin aineistoa muokattiin analyysikelpoiseksi yhtenäistämällä sen muuttujien koodausta. Analyysissä käytettiin Näkövammarekisteristä 1 831:n ja Retinitisrekisteristä 83:n potilaan tietoja. Rekisterien analyysi suoritettiin R- ja Microsoft Excel -ohjelmilla. Kirjallisuuskatsaus perustui elektronisten PubMed- ja MEDLINE-tietokantojen artikkeleihin.

Rekisterien perinnöllistä verkkokalvorappeumaa sairastavista suuri osa oli työikäisiä. Näkövammarekisterissä 50 % ryhmän miehistä oli 42–67-vuotiaita, naisista 49–71-vuotiaita. Retinitisrekisterissä 50 % tapauksista oli 38–51-vuotiaita. Näkövammarekisterin tapauksista 25 %:lla miehistä sairauden alkamisikä oli ≤17 vuotta, naisista ≤25 vuotta. Retinitisrekisterissä näköoireiden, erityisesti värinäön heikkenemisen, alkamisikä oli myös alhainen. Retinitis pigmentosa oli molemmissa rekistereissä hallitseva diagnoosi. Väestöön suhteutettu suurin perinnöllisen verkkokalvorappeuman prevalenssi oli Satakunnassa (0,051 %, n=114). Aineistoista vain Retinitisrekisteri sisälsi mahdollisen tiedon potilaan geenitestauksesta. Yleisimmät mutaatiot tässä rekisterissä olivat CERKL- ja EYS-geeneissä. Tällä hetkellä verkkokalvon rappeumasairauksiin ei ole korjaavaa hoitoa, mutta kantasoluihin perustuvia kliinisiä tutkimuksia on meneillään. Suomessa potilaiden geneettisten muutosten selvittäminen ja kirjaaminen kansallisiin rekistereihin edistäisi tuleviin tutkimuksiin soveltuvien potilaiden tunnistamista.

The originality of this thesis has been checked using the Turnitin OriginalityCheck service in accordance with the quality management system of the University of Tampere.

Tämän opinnäytteen alkuperäisyys on tarkastettu Turnitin OriginalityCheck -ohjelmalla Tampereen yliopiston laatujärjestelmän mukaisesti.

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# 1. Introduction

Hereditary retinal diseases affect retinal photoreceptor cells causing gradual, often progressive, loss of vision and visual impairment. In Finland, hereditary retinal diseases are the leading cause of visual impairment in working age population. These retinal diseases form a heterogeneous group including disorders which differ in pathophysiology, range of symptoms and disease progressing time. The combining element in the group is inheritance, and all known modes of inheritance are represented. (1,2.) Prevalence of hereditary retinal disease is about 1:1,000 worldwide, and there are about 5,000 patients in Finland (2).

Retinitis pigmentosa is the most common type of hereditary retinal disease with the prevalence of about 1:4,000 worldwide (3,4). Over 100 genes and their loci associating with retinitis pigmentosa have been identified. Phenotype of retinitis pigmentosa includes gradual loss of low-light (scotopic) vision as a result of rod cell degeneration, reduction of peripheral visual field, loss of visual acuity, dazzling and degenerative changes of fundus. (5,6.) Most of the gene mutations causing retinitis pigmentosa suppress selectively rod cell function. Defective gene product, e.g. phototransduction or membrane protein, can lead to rod cell apoptosis. The precise mechanisms these gene products act on rod function remain unclear. Following rod cell damage, cone cell function starts to impair in peripheral fundus region. (1,4.) This reduction of peripheral visual field is clinically seen as development of tunnel vision. The symptoms of retinitis pigmentosa depend on the mutated gene, but they vary widely because of the gene-environment interaction. At the end-stage, retinitis pigmentosa can lead to total blindness. The syndromic form of retinitis pigmentosa is associated with other ocular or systemic symptoms. Symptoms of Usher syndrome include hearing impairment and possible balance disorder, and Finnish heritage disease Cohen syndrome is associated with developmental disability and recognizable face features. (1,2.) The symptoms of retinitis pigmentosa can also be primarily caused by degeneration of cone cells, and thus these diseases are called cone-rod dystrophies (7).

The most severe retinal dystrophy is Leber congenital amaurosis. Its symptoms are caused by at least six gene mutations, of which RPE65 mutation is the best-studied. (8,9.) Genetic therapy for repairing RPE65 mutation has been performed on animals and humans (10).

Dystrophies of the macula are a class of hereditary retinal diseases that primarily affect the macula, central area of the eye responsible for contrast and colour vision. Apoptosis of rod cells on macular region cause dazzling, gradual loss of colour and contrast vision and visual acuity. (1)

Dystrophies of the macula include many identified diseases, one of which is Stargardt disease. Stargardt disease is inherited recessively in autosome and its onset is typically in young adulthood. Recognizable clinical signs of the disease are yellowish, usually fishtail shaped flecks on macula. Prevalence of Stargardt disease is estimated to be between 1:8,000 and 1:10,000 worldwide. (11.) These dystrophies of the macula are not to be mixed with age-related macular degeneration (AMD). AMD is the leading cause of visual impairment among elderly people (1). AMD is not considered as hereditary macular dystrophy, even though strong hereditary elements have been found related to it. Clinical sign of AMD is drusen, protein and lipid containing accumulation in subretinal space. Drusen causes retinal pigment epithelium cells (RPE cells) and finally the macula to degenerate, which leads to progressive vision loss. This kind of macular degeneration can proceed atrophically (dry AMD) or through scar formation following neovascularisation from the underlying choroid (wet AMD). (1,9.) Dry AMD covers approximately 85 % of all cases and there is no approved treatment for it. However, several therapy options, which include visual cycle modulators, neuroprotectants, anticomplement agents and drugs decreasing oxidative stress, are being studied. The choroidal neovascularisation (CNV) of wet AMD can be treated with intravitreous anti-VEGF agents (Avastin, Lucentis, Eylea) and thus slow down further vision loss. AMD is partly hereditary. (12.)

In addition to chemical treatment, cell therapy for degenerative macular diseases (Stargardt, AMD) is a promising option for tissue repair. RPE cells differentiated from human embryonic stem cells (hESC) and transplanted into human eye did not show adverse effects related to the injected cells in a study by Schwartz et al. (13). There are several ongoing preclinical and clinical studies on stem cell therapy for macular degenerational diseases (14). Gene-correction therapies are also being studied as independent treatments or as supportive options for stem cell therapy. Gene therapy with intravitreal AAV-sFLT01-injections (promoter for VEGF-neutralising protein in Adeno-Associate

viral vector) seemed to be well tolerated in a study by Heier et al. (15). Models using CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats - CRISPR associated proteins) gene editing tool for patient-derived induced pluripotent stem cells (iPSCs) have been studied preclinically (16). To date, there is no cure for retinal dystrophies, but some studies on stem cell and gene therapy have shown promising results on treating certain disease states.

Classification of hereditary retinal diseases is demanding as modern genetic study has revealed numerous genetic changes behind various clinical symptoms. The group is both clinically and genetically heterogeneous. (2.) The challenge is to survey both the prevalence of hereditary retinal diseases and their different genetic backgrounds in Finland. The survey is needed to assess the prevalence and variety of these diseases in Finland and target the patients with appropriate genetic or disease state for future research.

## 2. Materials and Methods

This observational, cross-sectional study was based on two national registers: Register of Visual Impairment, run by The National Institute for Health and Welfare, Finland (THL), and Register of Retinal Diseases, run by Retina Ry. Register of Visual Impairment is based on Finnish legislation and was established on 1 Jan 1983. It consists of information on c. 52,000 patients in Finland. (17.) In December 2015, 18,261 of the registrants were alive. Register of Retinal Diseases was established on 3 May 2016. There were 83 registrants in it on 24 May 2017. Statistical analyses of the registers were performed using Microsoft Excel and computing environment R (18,19).

Changes were made on Register of Retinal Diseases to enable statistical analysis process. Coding format of the register was modified in columns considering age of patients and reported diagnosis so that approximate, indefinite values were changed into definite values.

The literature review on hereditary retinal diseases, related gene mutations and stem cell therapies was conducted using electronical databases MEDLINE and PubMed.

# 3. Results

## 3.1. Register of Visual Impairment

Eight categories of Register of Visual Impairment were used in analysis: gender, age, registration year, onset year (year of diagnosis if unknown), onset age, diagnosis code (according to ICD-9, WHO)(20), classification of visual impairment (WHO) and place of residence (hospital district).

There were 18,261 living patients in the register in December 2015, of which 1,831 (10,03 %) had diagnosis for hereditary retinal disease.

The most prevalent diagnosis in the register was AMD (41 %). Other diagnoses in the original register were: visual tract defects (9 %), glaucoma (7 %), diabetic retinopathy (5 %), congenital developmental defects (5 %), undefined low vision (5 %), corneal defects (2 %), pathological myopia (2 %), visual field loss (2 %), retinal hole (2 %), choroidal defects (2 %) and other diagnoses (8 %). (17)

ICD-9 coding for hereditary retinal disease includes subclasses 362.70-362.79. There were 1,042 men (56,91 %) and 789 women (43,09 %) patients in this group. Men majority may have been present because of X-liked inheritance, which causes men to have greater possibility to represent the phenotype of the disease than women.

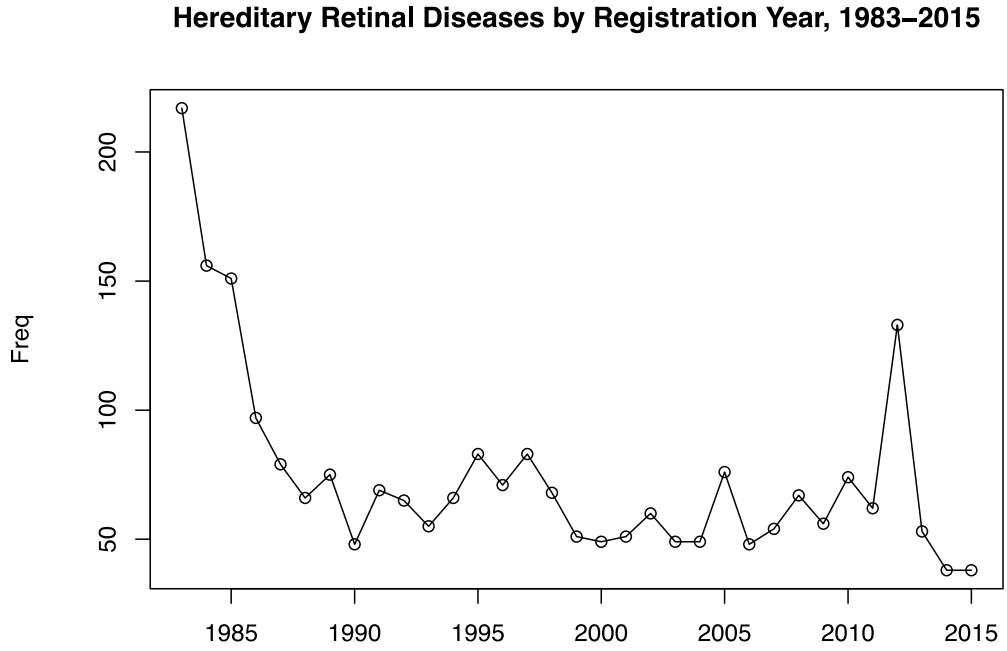


Figure 1. Frequency (n) of hereditary retinal diseases by registration year, 1983-2015.

The amount of registered hereditary retinal disease patients was high (266 patients) in 1983 when the register was established (figure 1). In 14 years' time, a downward trend can be seen in the frequencies of the registered patients, even though there was one noticeable peak in 2012 with 128 new registrants. The fewest new hereditary retinal disease patients were registered in 2014, 37 in total.

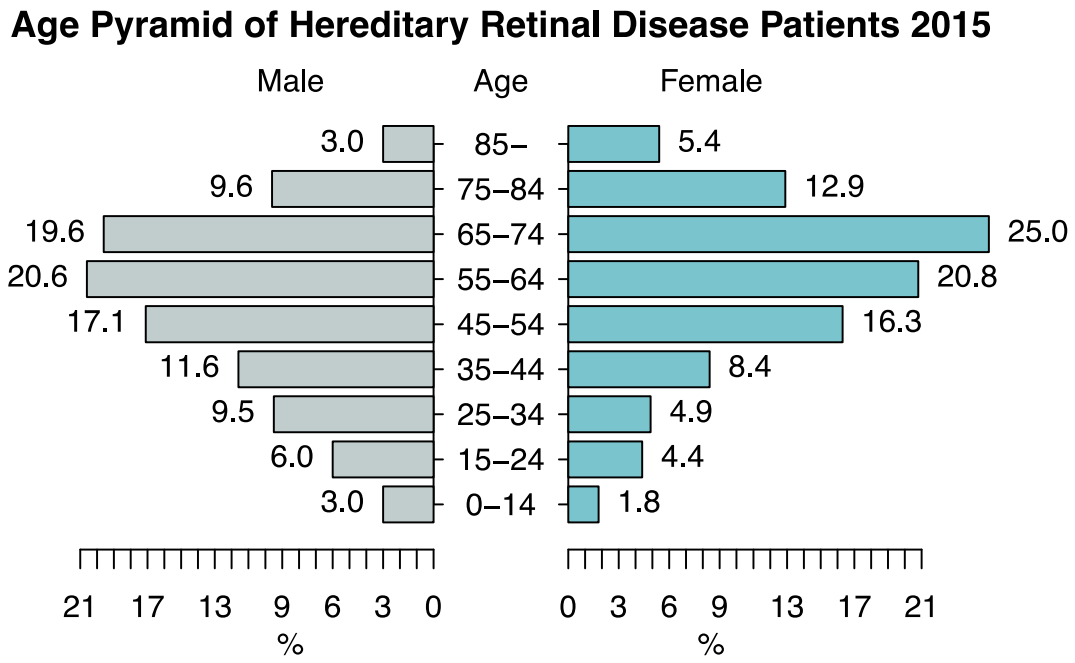


Figure 2. Age pyramid of the living hereditary retinal disease patients in 2015. Proportions in percentages (%) of all male (grey, n=1,042) and all female (blue, n=789) cases.

Age pyramid of the registrants (figure 2) shows the distribution of living female and male patients in age groups. Median age was 56,0 years for male and 62,0 years for female patients. Groups 55-64 and 65-74 were dominant in both sexes. Proportions of age groups below 35 years were greater in men than women: 18,5 % of the male and 11,1 % of the female patients were aged 0-34. The proportions of higher age groups differed between sexes: 32,2 % of the male and 43,4 % of the female patients were aged 65 or over. In men, lower quartile (25 %) was 41,0 years and upper quartile (75 %) 67,8 years. In women, corresponding values were 48,0 years and 71,0 years.

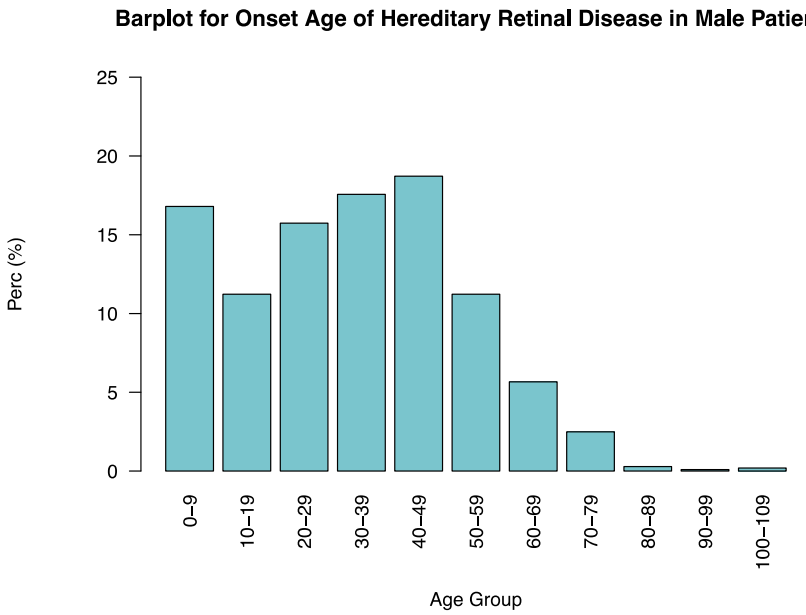


Figure 3. Bar plot for onset age of hereditary retinal disease in male patients. Proportions in percentages (%) of all male (n=1,  042) cases.

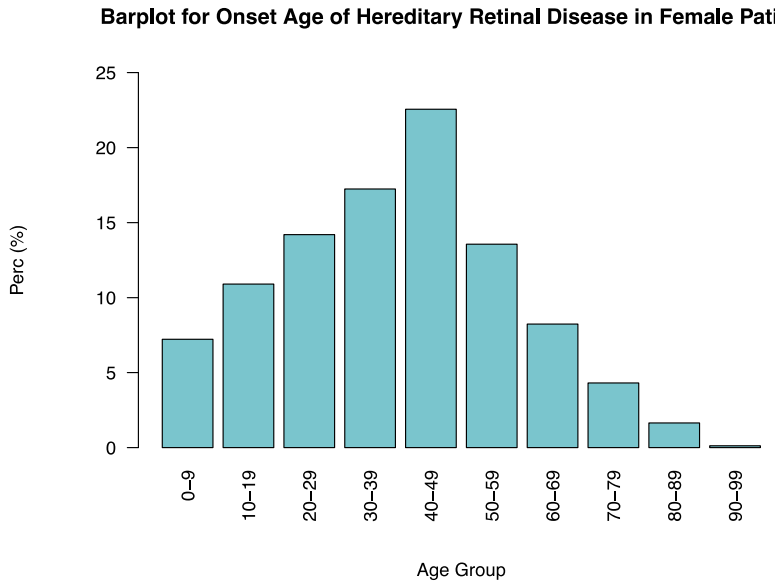


Figure 4. Bar plot for onset age of hereditary retinal disease in female patients. Proportions in percentages (%) of all female (n=789) cases.

Table 1. Frequency (n) and prevalence (%) of male hereditary retinal disease patients in onset age groups.

| Onset age group | n | Perc (%) |
|---|---|---|
| 0-9 | 175 | 16,79 |
| 10-19 | 117 | 11,23 |
| 20-29 | 164 | 15,74 |
| 30-39 | 183 | 17,56 |
| 40-49 | 195 | 18,71 |
| 50-59 | 117 | 11,23 |
| 60-69 | 59 | 5,66 |
| 70-79 | 26 | 2,5 |
| 80-89 | 3 | 0,29 |
| 90-99 | 1 | 0,1 |
| 100-109 | 2 | 0,19 |
| Total | 1042 | 100 |

Table 2. Frequency (n) and prevalence (%) of female hereditary retinal disease patients in onset age groups.

| Onset age group | n | Perc (%) |
|---|---|---|
| 0-9 | 57 | 7,22 |
| 10-19 | 86 | 10,9 |
| 20-29 | 112 | 14,2 |
| 30-39 | 136 | 17,24 |
| 40-49 | 178 | 22,56 |
| 50-59 | 107 | 13,56 |
| 60-69 | 65 | 8,24 |
| 70-79 | 34 | 4,31 |
| 80-89 | 13 | 1,65 |
| 90-99 | 1 | 0,13 |
| Total | 789 | 100 |

Hereditary retinal disease onset age bar plots for male and female patients differ. In male group, median age was 33,0 years, lower quartile (25 %) 17,0 years and upper quartile (75 %) 46,0 years. The proportions of age groups 0-9, 20-29, 30-39 and 40-49 years were almost equally dominant (figure 3, table 1). In female patients, median age was 40,0 years, lower quartile 25,0 years and upper quartile 51,0 years. Age group 0-9 years was less represented (16,79 % in men vs. 7,22 % in women) and the group 40-49 years had the largest proportion (22,56 %) (figure 4, table 2). The large proportion of low onset age male patients can be seen in the age pyramid of the registrants (figure 2). Both plots show that in higher age groups, after 70 years, the proportions become lower.

Table 3. Frequency (n) and prevalence (%) of hereditary retinal disease patients by hospital district.

| Hospital District | Population | Hereditary Retinal Disease Patients | Perc (%) |
|---|---|---|---|
| Satakunta | 224,556 | 114 | 0,051 |
| Lappi | 118,314 | 59 | 0,05 |
| Keski-Suomi | 250,773 | 124 | 0,049 |
| Pohjois-Savo | 248,43 | 109 | 0,044 |
| Ahvenanmaa | 28,666 | 12 | 0,042 |
| Pirkanmaa | 521,54 | 213 | 0,041 |
| Pohjois-Karjala | 169,112 | 67 | 0,04 |
| Kanta-Hame | 175,481 | 64 | 0,036 |
| Kainuu | 76,782 | 28 | 0,036 |
| Lansi-Pohja | 64,200 | 23 | 0,036 |
| Pohjois-Pohjanmaa | 403,555 | 132 | 0,033 |
| Etela-Savo | 104,407 | 34 | 0,033 |
| Kymenlaakso | 173,864 | 54 | 0,031 |
| Etela-Pohjanmaa | 198,831 | 59 | 0,03 |
| Vaasa | 168,848 | 50 | 0,03 |
| Ita-Savo | 44,444 | 13 | 0,029 |
| Varsinais-Suomi | 474,053 | 132 | 0,028 |
| Helsinki and Uusimaa | 1,581,450 | 433 | 0,027 |
| Paijat-Hame | 213,428 | 53 | 0,025 |
| Keski-Pohjanmaa | 78,284 | 18 | 0,023 |
| Etela-Karjala | 132,252 | 26 | 0,02 |

Table 3 shows the regional differences of the prevalence of hereditary retinal disease in Finland in 2015. Hospital district populations were based on calculations by Statistics Finland (Tilastotietokeskus) (21) and Association of Local and Regional Authorities (Suomen Kuntaliitto) (22). The patients living abroad (n=2) or with missing location data (n=12) were excluded from the table. The largest proportion of hereditary retinal disease patients lived in Satakunta (0,051 % of the population) and the smallest in Etelä-Karjala (0,020 % of the population).

Table 4. ICD-9 diagnoses, frequency (n) and prevalence (% of all cases, n=1,830) of hereditary retinal disease, and description by WHO (20).

| Diagnosis, ICD-9 | n | Perc (%) | Description |
|---|---|---|---|
| 362.70 | 299 | 16,34 | Unspecified hereditary retinal dystrophy |
| 362.71 | 37 | 2,02 | Retinal dystrophy in systemic or cerebroretinal lipidoses |
| 362.72 | 160 | 8,74 | Retinal dystrophy in other systemic disorders and syndromes |
| 362.73 | 226 | 12,35 | Vitreoretinal dystrophy |
| 362.74 | 909 | 49,67 | Pigmentary retinal dystrophy |
| 362.75 | 171 | 9,34 | Other dystrophies primarily involving the sensory retina |
| 362.76 | 25 | 1,37 | Dystrophies primarily involving the retinal pigment epithelium |
| 362.77 | 3 | 0,17 | Unspecified choroidal degeneration |
| Total | 1830 | 100 | |

Table 4 was calculated based on registrants' four columns of diagnoses. In those columns, overlapping diagnosis codes did not occur i.e. no patient represented more than one disease of subclasses 362.70-362.79. Official descriptions are made for subclasses 362.70-362.77 by WHO. One male patient with the code 362.78 was thus excluded. Table 4 shows that nearly half (49,67%) of the patients are diagnosed with pigmentary retinal dystrophy (retinitis pigmentosa).

## 3.2. Register of Retinal Diseases

### 3.2.1. Statistical analysis of Register of Retinal Diseases

Categories used in the analysis of Register of Retinal Diseases were gender, age, place of birth, diagnosis, status of genetic testing, disease appearance in family, additional eye disorders and onset age of specified visual disorder (loss of scotopic, colour and reading vision and vision for moving around). There were 42 male and 41 female patients in the register.

Table 5. Frequency (n) of hereditary retinal disease patients in age groups.

| Age group | n |
|---|---|
| 0-9 | 4 |
| 10-19 | 4 |
| 20-29 | 6 |
| 30-39 | 9 |
| 40-49 | 16 |
| 50-59 | 12 |
| 60-69 | 22 |
| 70-79 | 9 |
| 80-89 | 1 |
| **Total** | **83** |

The total distribution of the age groups was similar in Register of Retinal Diseases and Register of Visual Impairment. Groups between 40 and 70 were dominant (table 5, compare to tables 1 and 2). Median age was 51,0 years, lower quartile 37,0 years and upper quartile 51,0 years. However, there was no male majority seen in the group 0-19 years (3 men, 5 women).

Table 6. Hereditary retinal disease patients (n) by hospital district (place of birth).

| Hospital District | n |
|---|---|
| Helsinki and Uusimaa | 17 |
| Pirkanmaa | 8 |
| Varsinais-Suomi | 4 |
| Pohjois-Pohjanmaa | 12 |
| Keski-Suomi | 12 |
| Satakunta | 1 |
| Pohjois-Savo | 5 |
| Pohjois-Karjala | 2 |
| Kanta-Hame | 1 |
| Etela-Pohjanmaa | 2 |
| Lappi | 1 |
| Kymenlaakso | 0 |
| Paijat-Hame | 0 |
| Vaasa | 2 |
| Etela-Savo | 0 |
| Kainuu | 3 |
| Etela-Karjala | 2 |
| Lansi-Pohja | 2 |
| Keski-Pohjanmaa | 2 |
| Ita-Savo | 2 |
| Unknown | 2 |
| Abroad | 3 |
| **Total** | **83** |

Table 6 was formed based on the registrants' places of birth. Reported locations were reclassified into hospital districts and recoded. When compared with table 3 for places of residence in Register of Visual Impairment, majority of the registrants in Register of Retinal Diseases (20,5 %, n=17) were born in the hospital district of Helsinki and Uusimaa. The proportion of the patients born or living in Helsinki and Uusimaa region was approximately the same in both studied registers (23,6 % in Register of Visual Impairment). Between tables 3 and 6 there is a difference in the proportion of patients from Satakunta region: table 3 shows the high prevalence of hereditary retinal disease patients living in Satakunta (0,051 %), but in Register of Retinal Diseases, only one of the registrants is born there.

Table 7. Hereditary retinal disease diagnoses (n).

| Diagnosis | n |
|---|---|
| Retinitis pigmentosa | 34 |
| Retinitis pigmentosa, dominant | 6 |
| Retinitis pigmentosa, recessive | 22 |
| Retinitis pigmentosa, X-linked | 6 |
| Usher syndrome | 1 |
| Stargardt disease | 1 |
| Other macular degeneration | 1 |
| cone rod dystrophy | 6 |
| Vitelliform macular dystrophy | 1 |
| Choroidermia | 3 |
| Retinoschisis | 4 |
| Leber congenital amaurosis | 3 |
| Selective cone dystrophy | 1 |
| Hereditary retinal disease, unspecified | 2 |
| **Total** | **91** |

Table 8. ICD-9 diagnoses and frequency (n) of hereditary retinal disease, and description by WHO.

| ICD-9 | n | Description |
|---|---|---|
| 361.10 | 4 | Unspecified retinoschisis |
| 362.50 | 1 | Unspecified macular degeneration |
| 362.70 | 5 | Unspecified hereditary retinal dystrophy |
| 362.74 | 76 | Pigmentary retinal dystrophy |
| 362.75 | 1 | Other dystrophies primarily involving the sensory retina |
| 362.76 | 1 | Dystrophies primarily involving the retinal pigment epithelium |
| 363.55 | 3 | Choroideremia |
| **Total** | **91** | |

The diagnoses of the registrants were coded in two columns. To help to recognise disease groups, diagnoses (table 7) were recoded into ICD-9 codes (table 8). Major part of the registrants (91,57 %, n=76) was diagnosed with retinitis pigmentosa (ICD-9 code 362.74). The total number of diagnoses was higher (n=91) than the number of registrants (n=83) as some registrants had multiple diagnoses. The range of ICD-9 subclasses used in Register of Retinal Diseases was wider than in Register of Visual Impairment (compare table 8 with table 4). Codes 361.10 (unspecified retinoschisis), 362.50 (unspecified macular degeneration) and 363.55 (choroideremia) were found in addition to subclasses 362.70 and 362.74-362.77.

Table 9. Gene test status and frequency (n).

| Gene test | n |
|---|---|
| Done, result known | 20 |
| Not done | 45 |
| Done, result not known | 6 |
| Unknown | 12 |
| **Total** | **83** |

Table 10. Place of gene test and frequency (n) by hospital district.

| Hospital District | n |
|---|---|
| Helsinki and Uusimaa | 13 |
| Pohjois-Savo | 2 |
| Pohjois-Pohjanmaa | 5 |
| Other | 1 |
| Pirkanmaa | 6 |
| Varsinais-Suomi | 3 |
| **Total** | **30** |

54,2 % of the registrants in Register of Retinal Diseases were not genetically tested for their retinal disease (table 9). 26 of the patients were tested. 30 of the registrants announced their place of testing (table 10), and more than one third (n=13) of them had been tested in the Helsinki University Hospital. Almost all the patients were tested in public healthcare. Category ‘other’ included private testing clinics.

Table 11. Hereditary retinal disease appearance in family and frequency (n).

| Disease appearance in family | n |
|---|---|
| Yes | 44 |
| No | 17 |
| Unknown | 22 |
| **Total** | **83** |

44 of the registrants (53 %) were reported having relatives with the same diagnosis (table 11). 20 % (n=17) did not have relatives with the same diagnosis.

Table 12. Other eye disorders of the registrants and frequency (n).

| Other eye disorders | n |
|---|---|
| None | 38 |
| Cataract | 44 |
| Macular swelling | 4 |
| Other | 1 |
| Unknown | 1 |
| **Total** | **88** |

Table 13. Frequency (n) of patients in onset age groups of specified eye disorder.

| Onset Age | Low-Light Vision (n) | Colour Vision (n) | Reading Vision (n) | Vision for moving around (n) |
|---|---|---|---|---|
| 0-9 | 25 | 17 | 13 | 21 |
| 10-19 | 21 | 19 | 26 | 28 |
| 20-29 | 17 | 44 | 21 | 16 |
| 30-39 | 17 | | 21 | 16 |
| Unknown or No Symptoms | 3 | 3 | 2 | 2 |
| **Total** | **83** | **83** | **83** | **83** |

The table of other eye disorders of the registrants (table 12) was based on two different columns in the original register. Considering registrants' additional eye disorders, cataract was the major diagnosis (90 %, n=44). The total number of observations (n=88) was higher than the number of registrants (n=83) as 5 of the registrants had two additional diagnoses. 43 % (n=38) of the registrants did not have other eye disorders. The table 13 of specified eye disorders shows the frequencies of the patients (n) in certain onset age group. These disorders were noticed at young age: there were no reported onset ages above 39 years. 72 % of the registrants were aged 40 or over. Especially onset of colour vision loss identified with young age groups (all reported cases below 30 years).

### 3.2.2. Genetic changes reported in Register of Retinal Diseases

6 of the registrants in Register of Retinal Diseases were reported having CERKL gene mutation. CERKL was the most common mutation type in the register. There were 4 patients with EYS mutation. Among other mutation types tested there were USH3, GUCY2D, RetGC, MERTK, PRPF8, TULP1, RP1 and RS1.

# 4. Discussion

## 4.1. Genetic changes behind the diseases of Register of Retinal Diseases

Mutations in the CERKL (ceramide kinase-like) gene associate with retinal degenerative diseases, including cone-rod dystrophy, retinitis pigmentosa and AMD. The exact pathways mutations in this gene lead to photoreceptor cell apoptosis remain obscure, but recent studies have presented results which emphasise the meaning of CERKL gene products on protecting retinal cells under oxidative stress. CERKL seems to interact with mitochondrial antioxidant protein TRX2 (thioredoxin 2). By maintaining TRX2 in reduced form, CERKL products assist in regulation of the TRX2 antioxidant pathway. (23.) CERKL mutations seem to be enriched in Finnish population (24), which is in concordance with our findings.

EYS (eyes shut homolog) gene mutations link to autosomal recessive retinitis pigmentosa and autosomal recessive cone-rod dystrophy. On EYS knockout zebrafish models, mislocalisation of opsin proteins and other outer segment proteins and disruption of F-actin has been reported. Outer segment of the retina refers to the light absorbing parts of photoreceptor cells. These changes may be a cause of photoreceptor cell apoptosis. (25.)

Usher syndrome type 3 (USH3) has been associated to mutations in the Clarin 1 gene (CLRN1) (26). CLRN1 is by far the only gene discovered to relate to USH3 pathogenesis. Three different CLRN1 mutation types have been reported to appear in Finland by Västinsalo et al. (27). Cell culture studies have shown CLRN1 proteins to be located on plasma membrane, and mutations in this gene to cause abnormal protein localisation. The pathways through which CLRN1 mutations lead to the symptoms of retinitis pigmentosa are unknown. Globally, USH3 is the least common type of Usher syndrome forms 1-3. However, in Finnish population, USH3 covers more than 40 % of cases. (26.) Small Finnish settling population and isolation are thought to explain the abnormal mutation distribution (27).

GUCY2D (also known as RetGC) mutation is one of the mutations causing Leber congenital amurosis. TULP1 is a cytoplasmic, membrane-associated protein that is assumed to assists in transporting newly synthetised proteins towards outer segment of the retina. TULP1 mutations are linked to both retinitis pigmentosa and Leber congenital amaurosis. (28)

Mutations in MERTK, PRPF8 and RP1 genes cause retinitis pigmentosa. MERTK (MER Proto-Oncogene, Tyrosine Kinase) is a critical part of the phagocytosis regulator path in RPE cells (29). PRPF8 protein functions in pre-mRNA splicing (30). RP1 gene has been shown to mutate frequently and its mutations could be the most prevalent in both autosomal dominant and recessive retinitis pigmentosa (31).

Mutations in RS1 encoding gene are known to cause X-linked juvenile retinoschisis. RS1 is an extracellular adhesive protein and it is mostly secreted by photoreceptor and bipolar cells. (32.) X-liked retinoschisis is a part of Finnish disease heritage. The other form of the disease is degenerative, senile retinoschisis. The physiological cause of the disease is retinal separation into two layers. (33.)

## 4.2. Hereditary retinal disease cell modelling and therapy prospects

Reprogrammed human pluripotent stem cells (hiPSC) offer an accurate in vitro modelling tool for the mechanisms of hereditary retinal diseases. Gene editing tools (CRISPR/Cas9) will offer even more precise options for iPSC studies in generating patient derived, isogenic lines or inserting mutations in unaffected cells. (34)

Several hiPSC disease model lines have been created. One of the first hiPSC studies focused on Best disease and the effects of mutations in the BEST1 gene. (35.) Retinitis pigmentosa associated mutations in the PRPF31, USH2A (related to Usher syndrome), RP1, RP9, PRPH2, RHO, PEEP6, MERTK and RP2 genes have been studied in hiPSC cultures (36-41). Leber congenital amaurosis causing mutations in the CEP290 and RPE65 genes have also been described (42,43). In addition to traditional hereditary retinal diseases, AMD hiPSC lines have been created (44,45). In comparison to the mutations found in Register of Retinal Diseases, only MERTK and RP1 hiPSC lines have been studied of the nine mutation types described in our study. hiPSC with RP1 mutation have showed significant loss of rhodopsin containing rod cell formation (38) and MERTK mutations have been confirmed to cause defect in phagocytosing the outer segment (OS) (40). Considering the recent findings on the high prevalence of CERKL mutations in Finland (24) there is a need for better understanding of these mutations in vitro.

Stem cells have also shown to be potential on developing therapy options for hereditary retinal diseases. Especially iPSC and hESC derivatives seem suitable for human eye applications. The intended outcome of regenerative therapy with stem cell-derived RPE or photoreceptor cells is to replace damaged native cells and restore their function. (46)

The latest studies have shown that injected hESC-derived RPE cells are well tolerated in AMD and Stargardt diasease patients over a period of 37 months (47). There were no severe adverse effects attributed to transplantation in the study cited. Even though the eye is considered as an immunologically tolerant organ, patients' own, autologous iPSC-derivatives might have the advantage of reducing the risk of post-transplantation inflammation. In addition, degenerative

retinal diseases suppress blood-retinal barrier function by damaging the tight RPE cell junctions and thus weaken the immunological protection. (48.)

Genetic editing tools such as CRISPR/Cas, ZFNs (zinc finger nucleases) and TALENs (transcription activator-like effector nucleases) might improve the safety and effectiveness of stem cell therapies in the future. Combining stem cell therapy with genetic editing would direct therapy development towards targeted treatments. (48)

To date, there are no ongoing clinical trials on retinal stem cell therapies in Finland.

## 4.3. Statistical analysis and coding

The coding format of both Register of Visual Impairment and Register of Retinal diseases played an important role in running statistical analysis. As a register with history of over 30 years, the coding format and reporting style of Register of Visual Impairment was standardised and coherent. Thus, importing its data into computing environment R was possible without data modification. This improves the validity of the results. The original data of Register of Retinal diseases was not fully suitable for importing without recoding. Subjective and approximate values (e.g. onset age at teenager, at 20-23) were recoded into numerical values (onset age at 13, at 20) to standardise the coding format and make the two registers comparable. Thus, informational bias may be present in the results. The modified values have an inevitable subjective tone which reduces the validity of the results.

The great number of living registrants in Register of Visual Impairment (n=1,831) made the data suitable for statistical analysis. On the contrary, figures were an undesirable way to visualise the data of Register of Retinal Diseases as the number of patients was relatively low (n=83). Because of this, the analysis of the data of Register of Retinal Diseases was presented on tables.

## 4.4. Main results and future research

Both analysis on Register of Visual Impairment and Register of Retinal Diseases showed similar results considering the age of hereditary retinal disease patients. In Register of Visual Impairment, 50 % of the male patients were aged 42-67 years and the corresponding ages for female patients were 49-71. In Register of Retinal Diseases 50 % of all registrants were aged 38-51. Thus, the high prevalence of working age registrants was seen in both registers. In addition, the incidence of hereditary retinal diseases and the eye symptoms related to them was high in young age groups: in Register of Visual Impairment, 25 % of the male patients had disease onset age ≤17 years. 25 % of the female patients had onset age ≤25 years. In Register of Retinal Diseases, the reported onset ages of colour vision loss were all below 30 years.

Retinitis pigmentosa was a predominant diagnosis in both studied registers with prevalence of nearly 50 % (Register of Visual Impairment) and 92 % (Register of Retinal Diseases). In Register of Visual Impairment, 16 % of the registrants had unspecified diagnosis. Difficulties in identifying these diseases may be caused by clinical similarities between hereditary retinal dystrophies and lack of suitable genetic testing. The prevalence of unspecified diagnoses was not as high in Register of Retinal Diseases. In addition, the different types of retinitis pigmentosa were reported separately in this register. The most prevalent type was autosomal recessive retinitis pigmentosa (27 %, n=83).

The studied registers are not fully comparable to each other considering the geographical distribution of the diseases because of the different units used (place of residence vs. place of birth). In Register of Visual Impairment, the highest prevalence of hereditary retinal disease patients was in Satakunta (0,051 %, n=114) and lowest in Etelä-Karjala (0,02 %, n=26). The number of patients was highest in Helsinki and Uusimaa (n=433) and Pirkanmaa (n=213) regions.

Genetic testing status was not reported in Register of Visual Impairment. In Register of Retinal Diseases, 54 % of the patients were not genetically tested. The prevalence of disease appearance in family was 53 %. The modes of inheritance enable retinal diseases to strongly affect descendants or, on the other hand, skip several generations ineffective. In addition, approximately 50 % of the retinitis pigmentosa cases are isolated i.e. they are diagnosed in patients without any family history

of the disease (49). These may explain rather large proportion of those registrants (20 %, n=17) who were not reported having their disease appearing in the family. The genetic data on mutations in Register of Retinal Diseases was reported with open questions and thus partly inadequate. However, 6 CERKL and 4 EYS gene mutation cases were found.

In Finnish population, hereditary retinal diseases cause early onset visual impairment. Especially young male patients are affected. To date, there are no regenerative therapy options for retinal degenerative diseases. Ongoing clinical trials for treating glaucoma, AMD or RP are performed in USA, Russia, Korea, Israel, China and UK (50). Knowledge of the genetic mutations behind these diseases and their prevalence in Finland is still insufficient, and the pathological pathways partly undiscovered. Future research based on wide and precise genetic mapping and accurate coding into the national register systems could help to identify suitable cases for further in vitro and clinical studies.

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# 7. Appendices

Table 14. Visual impairment classification, frequency (n), prevalence (%) of all cases (n=1,831) and description by WHO in Register of Visual Impairment.

| Classification for visual impairment (WHO) | n | Perc (%) | Description |
|---|---|---|---|
| 0 | 72 | 3,93 | Mild or no visual impairment |
| 1 | 759 | 41,45 | Moderate visual impairment |
| 2 | 194 | 10,6 | Severe visual impairment |
| | | | |
| 3 | 386 | 21,08 | Blindness |
| 4 | 365 | 19,93 | Blindness |
| 5 | 24 | 1,31 | Blindness |
| | | | |
| 9 | 31 | 1,69 | Undetermined or unspecified |
| Total | 1,831 | 100 | |